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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Insight into odorant perception: the crystal structure and binding characteristics of antibody fragments directed against the musk odorant traseolide.

Langedijk AC, Spinelli S, Anguille C, Hermans P, Nederlof J, Butenandt J, Honegger A, Cambillau C, Pluckthun A.

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Monoclonal antibodies were elicited against the small hydrophobic hapten traseolide, a commercially available musk fragrance. Antibody variable region sequences were found to belong to different sequence groups, and the binding characteristics of the corresponding antibody fragments were investigated. The antibodies M02/01/01 and M02/05/01 are highly homologous and differ in the binding pocket only at position H93. M02/05/01 (H93 Val) binds the hapten traseolide about 75-fold better than M02/01/01 (H93 Ala). A traseolide analog, missing only one methyl group, does not have the characteristic musk odorant fragrance. The antibody M02/05/01 binds this hapten analog about tenfold less tightly than the original traseolide hapten, and mimics the odorant receptor in this respect, while the antibody M02/01/01 does not distinguish between the analog and traseolide. To elucidate the structural basis for the fine specificity of binding, we determined the crystal structure of the Fab fragment of M02/05/01 complexed with the hapten at 2.6 A resolution. The crystal structure showed that only van der Waals interactions are involved in binding. The somatic Ala H93 Val mutation in M02/05/01 fills up an empty cavity in the binding pocket. This leads to an increase in binding energy and to the ability to discriminate between the hapten traseolide and its derivatives. The structural understanding of odorant specificity in an antibody gives insight in the physical principles on how specificity for such hydrophobic molecules may be achieved.

PMID: 10525411 [PubMed - indexed for MEDLINE]

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Comparison of a fungal (family I) and bacterial (family II) cellulose-binding domain.

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A family II cellulose-binding domain (CBD) of an exoglucanase/xylanase (Cex) from the bacterium Cellulomonas fimi was replaced with the family I CBD of cellobiohydrolase I (CbhI) from the fungus Trichoderma reesei. Expression of the hybrid gene in Escherichia coli yielded up to 50 mg of the hybrid protein, CexCBDCbhI, per liter of culture supernatant. The hybrid was purified to homogeneity by affinity chromatography on cellulose. The relative association constants (Kr) for the binding of Cex, CexCBDCbhI, the catalytic domain of Cex (p33), and CbhI to bacterial microcrystalline cellulose (BMCC) were 14.9, 7.8, 0.8, and 10.6 liters g-1, respectively. Cex and CexCBDCbhI had similar substrate specificities and similar activities on crystalline and amorphous cellulose. Both released predominantly cellobiose and cellotriose from amorphous cellulose. CexCBDCbhI was two to three times less active than Cex on BMCC, but significantly more active than Cex on soluble cellulose and on xylan. Unlike Cex, the hybrid protein neither bound to alpha-chitin nor released small particles from dewaxed cotton fibers.

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1: J Chromatogr B Biomed Sci Appl 1998 Sep 11;715(1):283-96

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Characterization and affinity applications of cellulose-binding domains.

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Cellulose-binding domains (CBDs) are discrete protein modules found in a large number of carbohydrolases and a few nonhydrolytic proteins. To date, almost 200 sequences can be classified in 13 different families with distinctly different properties. CBDs vary in size from 4 to 20 kDa and occur at different positions within the polypeptides; N-terminal, C-terminal and internal. They have a moderately high and specific affinity for insoluble or soluble cellulosics with dissociation constants in the low micromolar range. Some CBDs bind irreversibly to cellulose and can be used for applications involving immobilization, others bind reversibly and are more useful for separations and purifications. Dependent on the CBD used, desorption from the matrix can be promoted under various different conditions including denaturants (urea, high pH), water, or specific competitive ligands (e.g. cellobiose). Family I and IV CBDs bind reversibly to cellulose in contrast to family II and III CBDs which are in general, irreversibly bound. The binding of family II CBDs (CBD(Cex)) to crystalline cellulose is characterized by a large favourable increase in entropy indicating that dehydration of the sorbent and the protein are the major driving forces for binding. In contrast, binding of family IV CBDs (CBD(N1)) to amorphous or soluble cellulosics is driven by a favourable change in enthalpy which is partially offset by an unfavourable entropy change. Hydrogen bond formation and van der Waals interactions are the main driving forces for binding. CBDs with affinity for crystalline cellulose are useful tags for classical column affinity chromatography. The affinity of CBD(N1) for soluble cellulosics makes it suitable for use in large-scale aqueous two-phase affinity partitioning systems.

PMID: 9792516 [PubMed - indexed for MEDLINE]